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A PRELIMINARY INVESTIGATION OF TUMOR TAKE INHIBITORY ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *MURRAYA KOENIGII* LINN. IN MICE

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ABSTRACT: The present study was undertaken to explore the tumor take inhibitory effects of ethanolic extract of *Murraya koenigii* in rodents. Tumor take inhibitory activity was investigated in hybrid mice (of C57BL strain + Swiss albino strain). Preventive group animals were injected daily with the extract at dose of 50mg/kg body weight i.p. for 10 consecutive days. The animals were observed for the growth of tumor after injection of B16F10 melanoma cells into the dorsal skin of mice. Pretreatment with the extract and showed delay tumor growth by increasing the volume doubling time, VDT ($p<0.01$), growth delay, GD ($p< 0.01$) and mean survival time, MST ($p<0.001$). Tumor regression studies showed a regression response for tumor growth *in vivo* of murine mouse melanoma tumor cell lines, demonstrated by increasing the VDT and GD.

INTRODUCTION: In a mature animal, a balance is usually maintained between cell renewal and cell death in most organs and tissues. The various types of mature cells in the body have a given life span; as these cells die; new cells are generated by the proliferation and differentiation of various types of stem cells [1]. Under abnormal conditions, the cells give rise to clones of cells that can expand to a considerable size, producing a tumor, or neoplasm [2]. Tumors are caused by mutations in DNA of cells. *Murraya koenigii* L. (Rutaceae) is commonly known as Meethi Neem. In India, it is used as a flavouring agent in curries and chutneys. The leaves of the curry tree are used as an important herb in the medical science of Ayurveda.

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They are believed to have remarkable glucose reducing effect for patients suffering from the sugar disease. The leaves, the roots and the bark of the curry tree can be used as tonics as well as a stomachic. The roots and the bark are stimulants suggested widely by herbalists. A mixture made from the curry tree is applied on external wounds and is known to relieve bites of poisonous animals.

The leaves of *Murraya koenigii* are eaten raw for relieving vomiting and dysentery. The oil made of the curry tree leaves and its seeds have been researched upon. This essential oil is said to contain antifungal and antibacterial properties [3]. It was reported to exhibit anti-inflammatory [4], analgesic [5], immunomodulatory [6], lipid lowering [7], wound healing [8], antidiabetic [9], anti-ulcer [10] and anti-oxidant activity [11]. *Murraya koenigii* is dominated by carbazole alkaloids, essential oil and carotenoids [12 - 14].

This study provides scientific evidence for the application of the traditional medicinal plant,

Murraya koenigii, in the retardation of tumor growth.

METHODS:

Plant Material: The leaves of *Murraya koenigii* linn. were collected from Bhopal, Madhya Pradesh, India and were identified and authenticated by Dr. Zia ul Hassan, Assistant professor, Department of Botany, Saifia College of Science and Education, Bhopal. A voucher specimen No.175/Bot/Safia/ 2010 is deposited in the herbarium of botany department.

Extraction: The dried drug (750 g) was coarsely powdered and then exhaustively extracted with 90% ethanol in soxhlet apparatus. The ethanolic extract so obtained was freed of solvent under vacuum to get 92 g (13.14% yield) of dark greenish brown mass.

Screening for Tumor take Inhibitory Activity:

Animal Model: The hybrid mice (of C57BL strain + Swiss albino strain) was selected, from a random breed colony maintain in the animal house of Research Department of Jawaharlal Nehru Cancer Hospital, Bhopal, Madhya Pradesh, India. The mice were housed in polypropylene cages maintained under controlled conditions. The animals were fed standard feed (formula obtained from Cancer Research Institute, Mumbai, India) and acidified water *ad libitum*. Mice of either sex, 6 - 8 weeks old and weighing 20 - 25 g, were selected from the above colony for the experiments.

Tumor Model: B16F10 Melanoma: B16F10 melanoma originally obtained from National Cell Centre, Pune, India, was maintained by serial transplantation in C57BL mouse.

Tumor Propagation: Tumor bearing mouse was sacrificed by cervical dislocation and the whole animal was dipped in 70 % alcohol and the tumor was excised to single cell suspension by mechanical dispersion. The cell suspension was filtered through a 45 µ nylon mesh. The single cell suspension was then passed through different gauze size needle. The cell suspension was again passed through nylon mesh so as to remove the clumps of cells.

Methodology: The animals were divided into two groups. Group I served as control and Group II served as test group. Group II was injected daily with the ethanolic leaf extract of *Murraya koenigii* 50mg/kg body wt. i.p. for 10 consecutive days. Three weeks after the last injection of the extract, the animals were injected with 5 x 10^5 viable B16F10 melanoma cells into the dorsal skin. The animals were observed for the growth of tumor. Volume doubling time and Growth delay were calculated [15 - 16].

Tumor Growth Kinetics: The tumor size was measured every alternate day and the tumor volume was calculated. Tumor diameters are measured with digital callipers, and the tumor volume in mm^3 is calculated by the formula: volume = $(width)^2$ x length/2.

Tumor growth response was assessed from the following parameters:

Volume doubling time (VDT): The time, in days for the tumors size to reach double the treatment volume.

Growth delay (GD): Difference in the time, in days, needed for the treated and untreated tumor to reach five times the treatment volume.

Statistical Analysis: Statistical evaluation of the data was done by Student‘t’ test. (Graph PAD Instat software, Kyplot). A value of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION: Pretreatment with the test extract showed delay tumor growth, demonstrated by growth curve, by increasing the Volume Doubling Time (VDT) and Growth Delay (GD) **Fig. 1**.

Silent Period: The silent period (*i.e.* time taken for palpable growth) for the control group was found to be 1 day while in case of ethanolic extract treated group, it was found to be 5 days respectively, which was very significant ($p <0.001$).

Volume Doubling Time: The volume doubling time observed for ethanolic extract was found to be 3 days that was significant ($p<0.01$) compared to control.

Growth Delay: The GD was 2 days in ethanolic extract treated group, which was significant ($p<0.01$) compared to control **Table 1**.

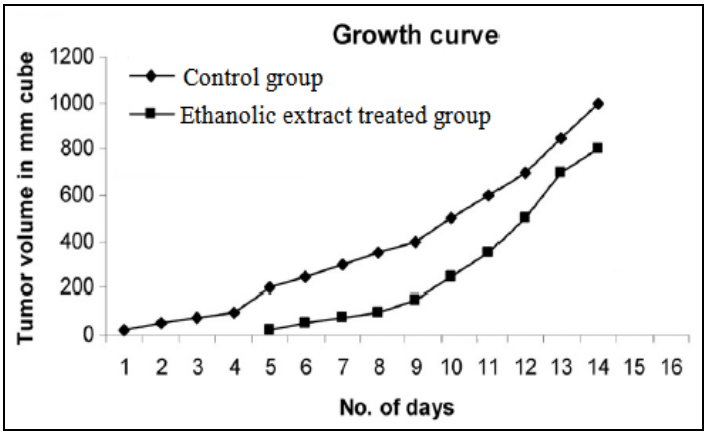

FIG. 1: GROWTH CURVE

TABLE 1: RESPONSE OF B16F10 MOUSE MELANOMA TO THE TREATMENT WITH ETHANOLIC EXTRACT AT THE DOSE OF 50 mg / kg: SILENT PERIOD, VDT, GD AND MST

Parameter	Control	Ethanolic Extract Treated Group
Silent Period	01 ± 0.35	05±0.45 [a]
Volume doubling time (VDT) days	01 ± 0.10	03± 0.31 [b]
Growth delay (GD) days	-	02±0.22 [b]
Mean Survival Time (MST)	24 ± 0.75	29±0.73 [a]

Values are expressed as mean ± S.E.M. for six animals in each group.
[a]: $p<0.001$ [b]: $p<0.01$ compared to control.

Mean Survival Time: The maximum survival time was observed to be 24 days for control group. The MST observed for ethanolic extract treated groups was 29 days, which was 5 days more than the control group. Comparisons in between MST of control group with test drugs treated group were found to be very significant ($p < 0.001$). Tumor regression studies showed a regression response for tumor growth *in vivo* of a murine mouse melanoma. The treatment produced a delay in tumor growth, as demonstrated by increasing the VDT and GD. Indications are available that this plant has got antioxidant properties. Oxidative stress has been implicated in numerous pathophysiological conditions including cancer.

Research on herbal medicines is gaining ground and the demand to use natural products in the treatment of various disorders is increasing worldwide. Tumor regression studies showed a regression response for tumor growth in vivo of a murine mouse melanoma. Tumor regression was related to the immune-stimulatory properties of the antibody [17]. The treatment produced a delay in tumor growth, as demonstrated by increasing the VDT and GD. Indications are available that this plant has got antioxidant properties. Oxidative stress has been implicated in numerous pathophysiological conditions including cancer. Prevention of oxidative damage can be employed as one of the ways in tumor regression.

CONCLUSION: Treatment with *Murraya koenigii* leaf extract showed a tumor regression response. The present investigations may be quite useful as this drug is highly valued as traditional system of medicine.

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CONFLICTS OF INTEREST: Nil.

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